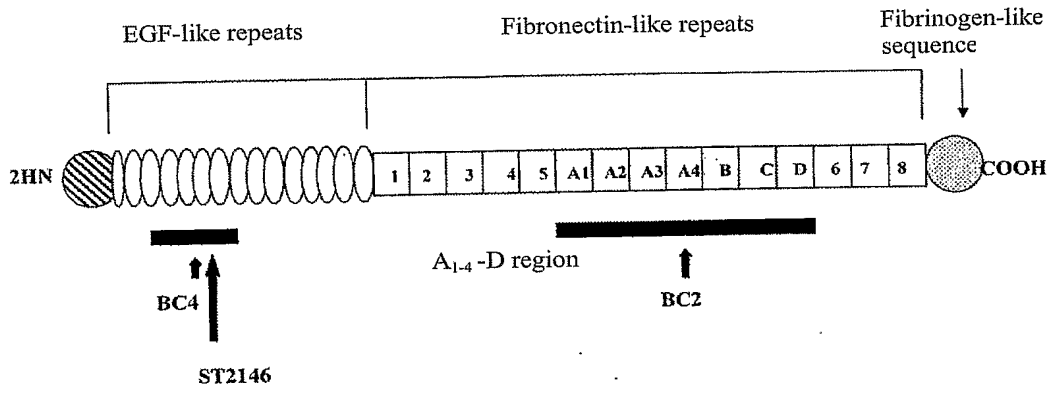
Figure 1

Schematic representation of human tenascin-C and of the strategy followed for the generation of BC2-like antibodies



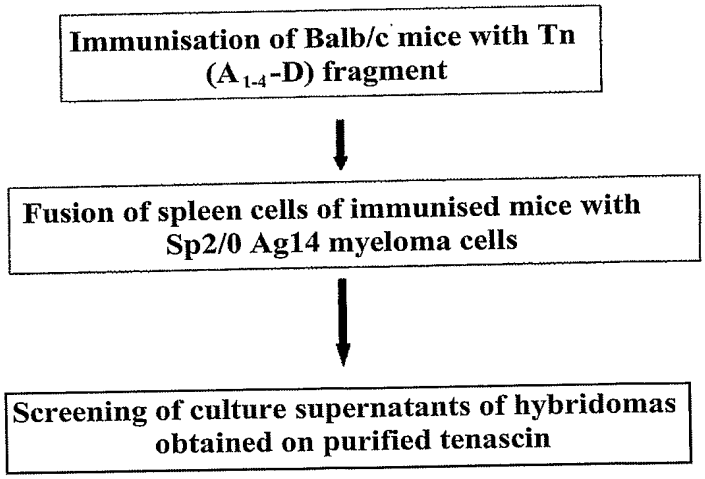
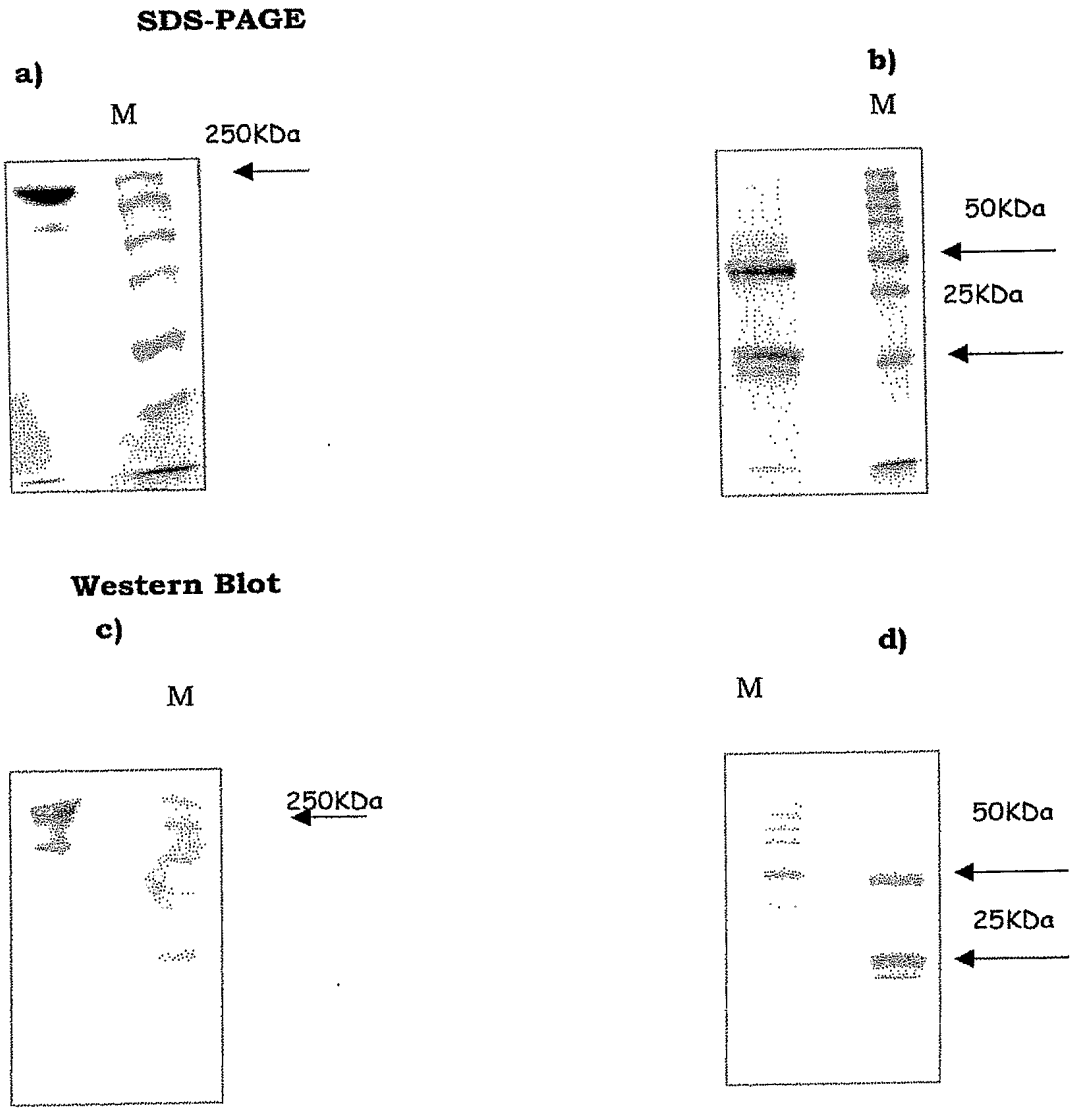


Figure 2

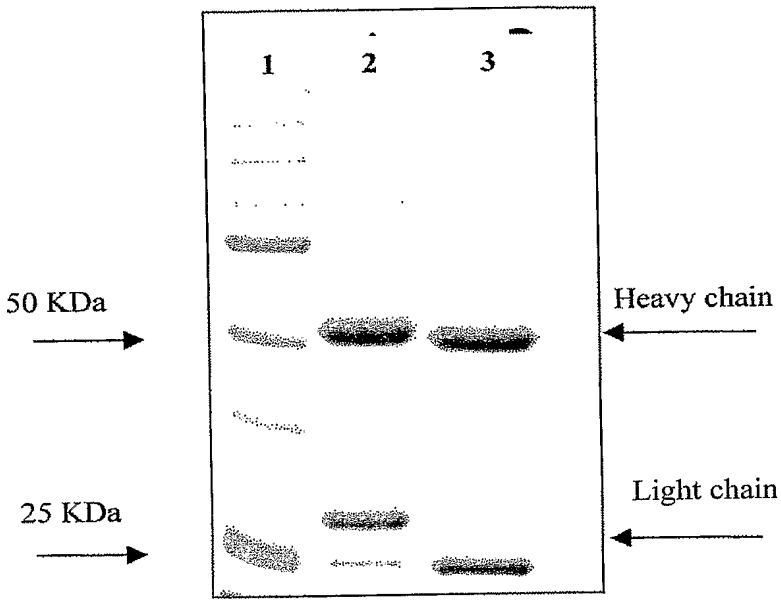
SDS-PAGE and Western Blot analysis of ST2485 antibody, in reducing (b, d) and non-reducing (a, c) conditions.



M: molecular weight standards

Figure 3

ST2485 antibody digestion with Flavobacterium Peptide-N-glycosidase enzyme (PNGase F).



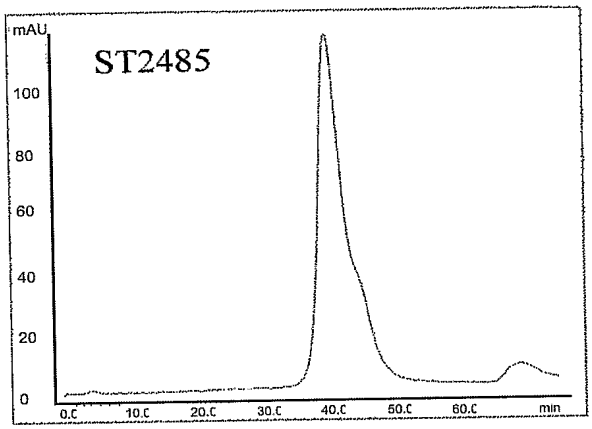
1: Molecular weight marker

2: Non-digested ST2485

3: PNGaseF-digested ST2485

Figure 4

BC-2 and ST2485 antitenascin antibodies hydroxyapatite chromatography.



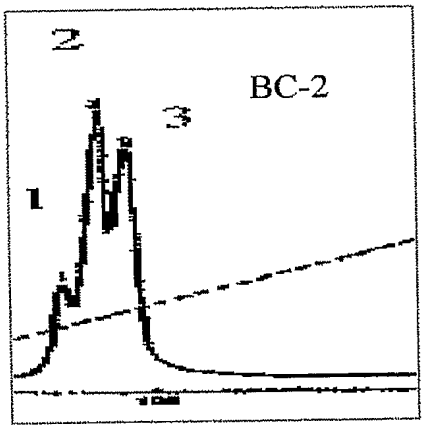
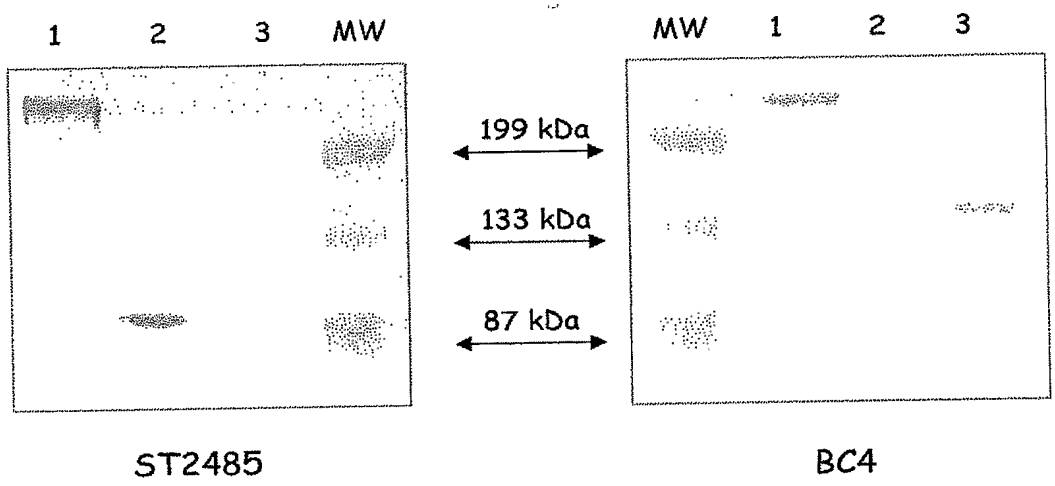


Figure 5

ST2485 antitenascin antibody Western Blot Analysis



1: Tenascin-C

2: Tn $A_{(1-4)}$-D Fragment

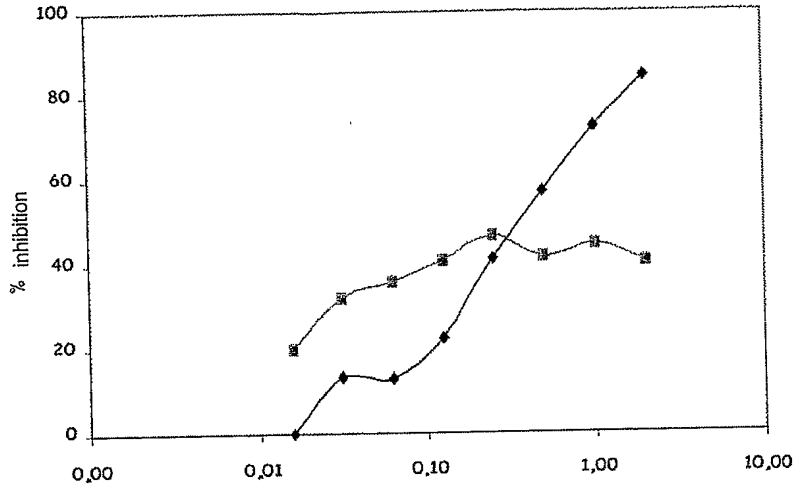
3: Tenascin EGF-like region recombinant fragment, containing the epitope recognized by BC-4 antibody.

MW: molecular weight standards

Figure 6

Competitive ELISA test between ST2485 and BC-2 for antigen binding.

a)



b)

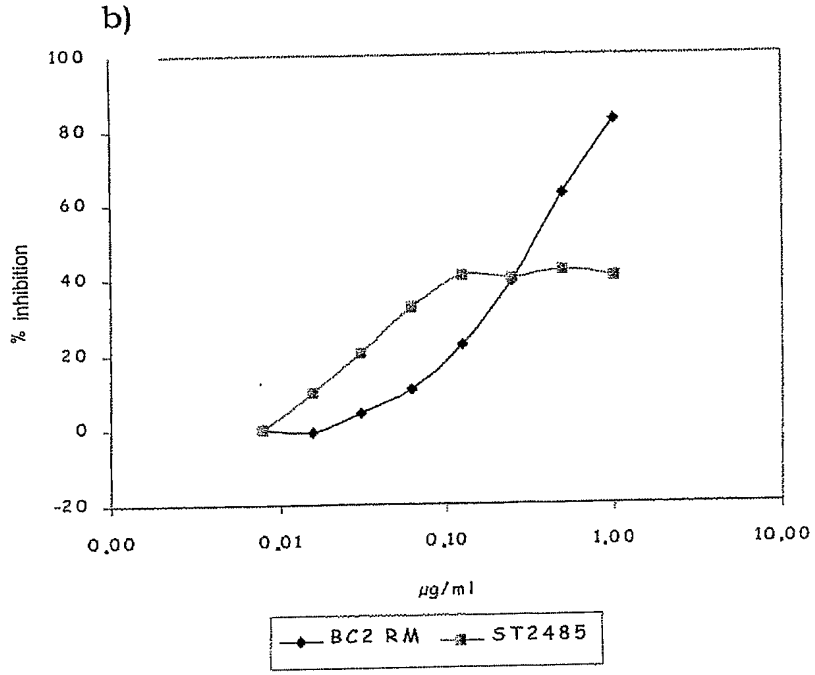
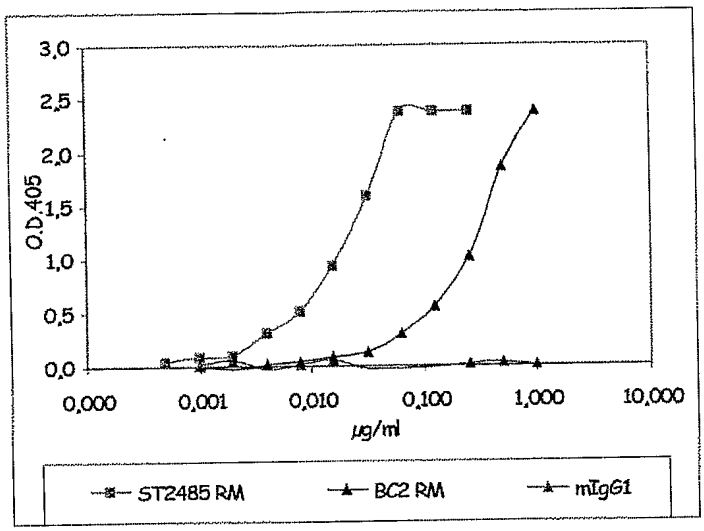


Figure 7

Immunoreactivity of ST2485 antibody in comparison with BC-2, on tenascin C (a) and on Tn $A_{(1-4)}$-D fragment (b).

a)



b)

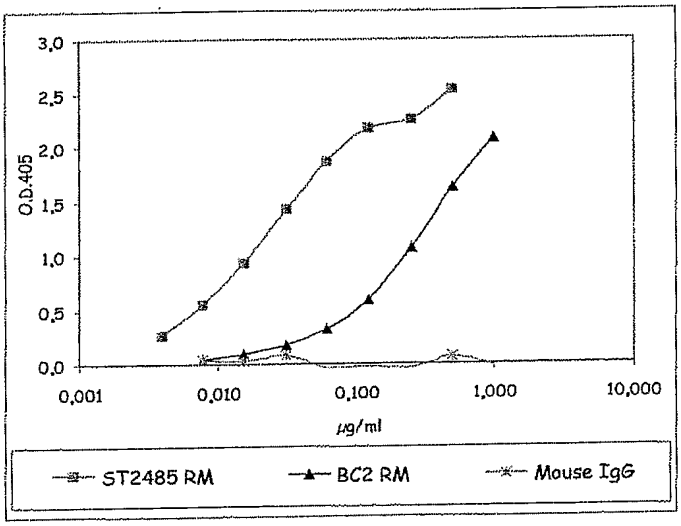
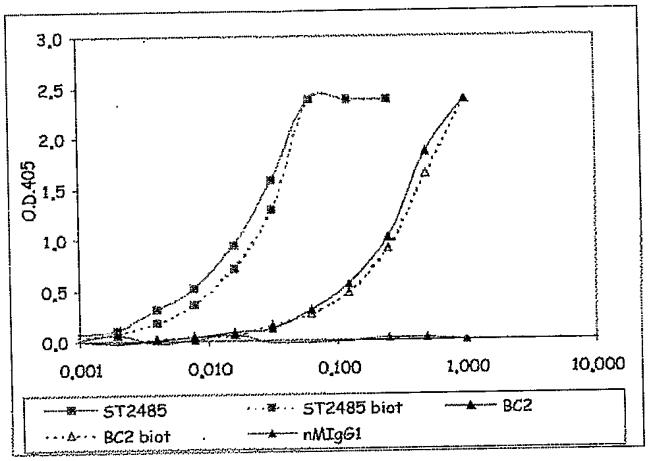
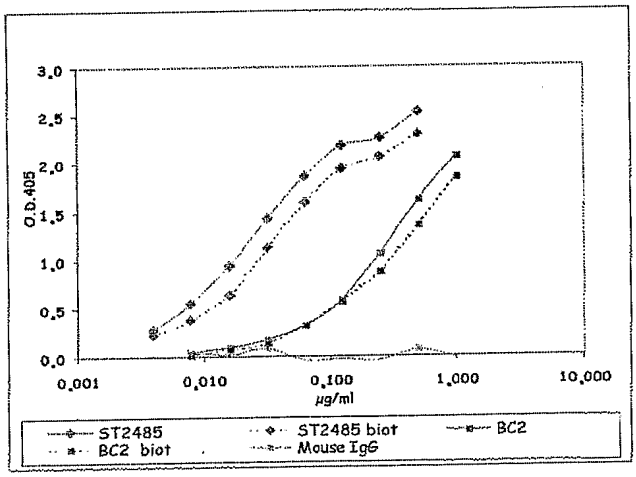


Figure 8

Immunoreactivity of ST2485 and BC-2 biotinylated and non-biotinylated antibodies, on tenascin-C (a) and on Tn $A_{(1-4)}$-D fragment (b).



a)



b)

Figure 9

Cross-reactivity of ST2485 antibody with murine tenascin.

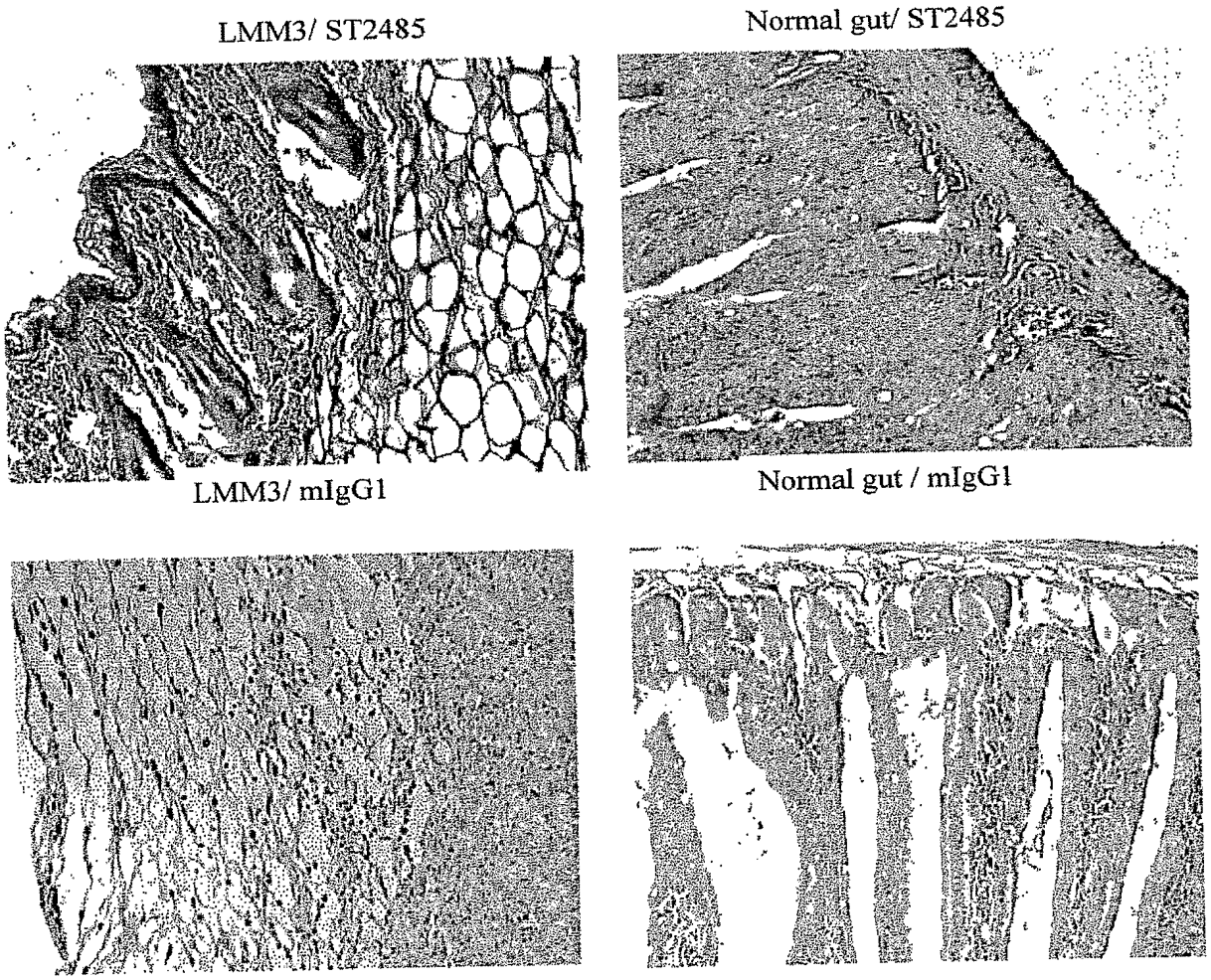


Figure 10

Biotinylated ST2485 and BC-2 antibodies biodistribution study protocol in human tenascin-expressing tumor-implanted nude mice.

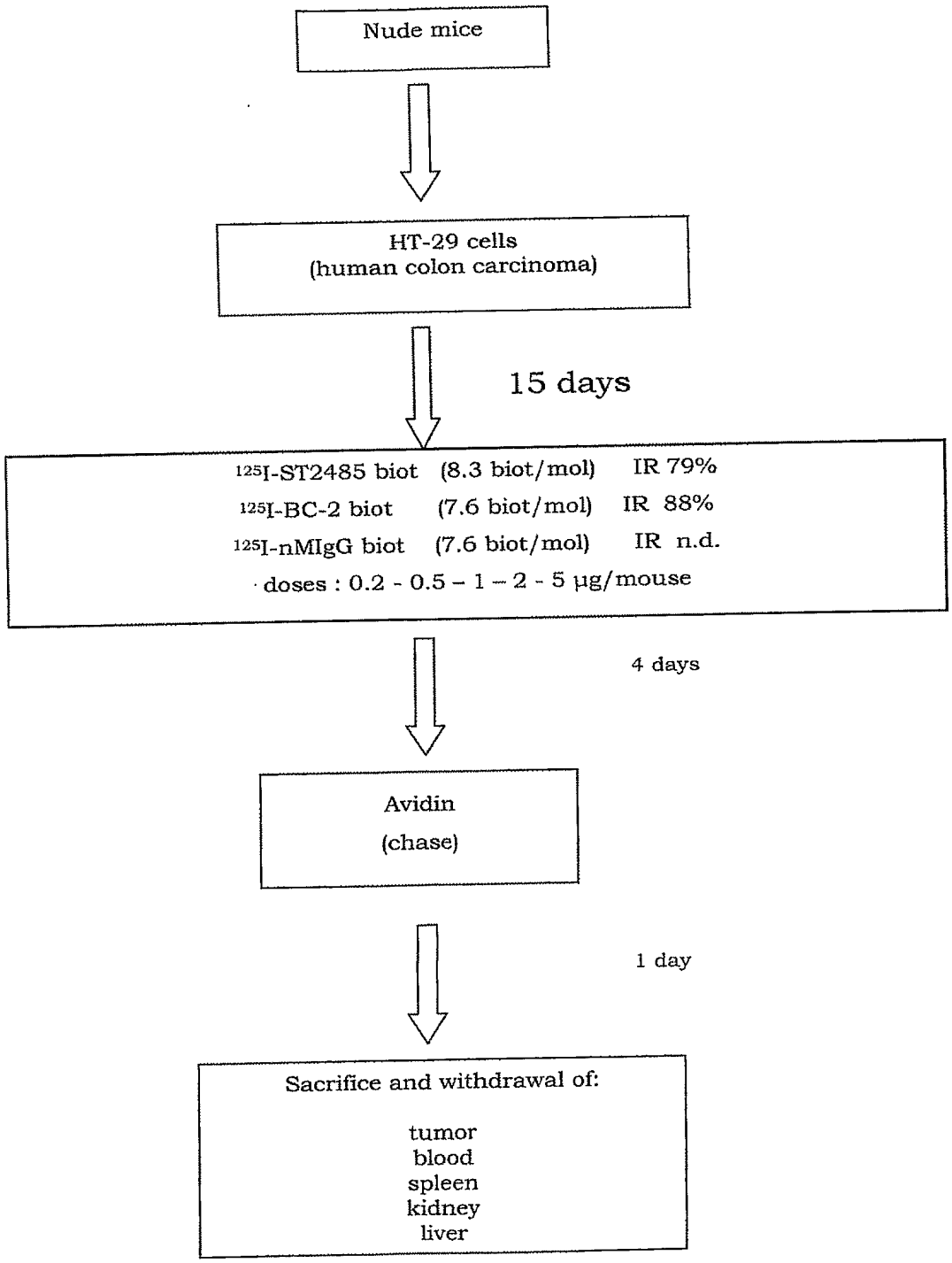
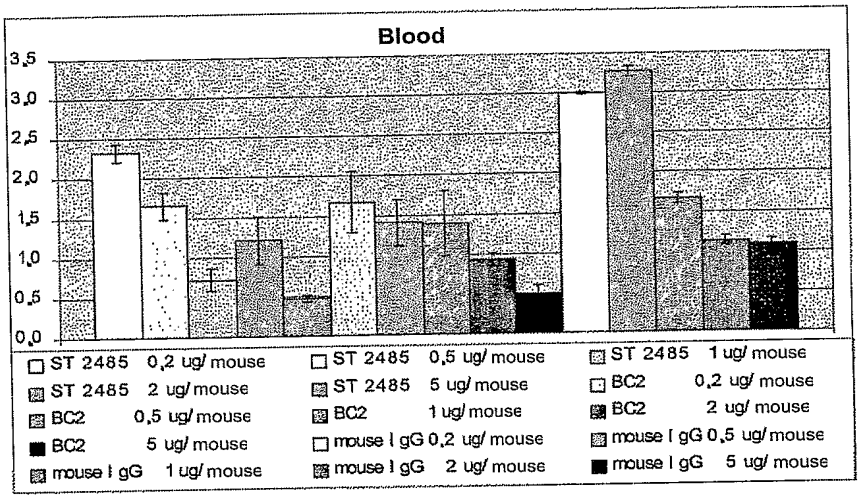


Figure 11

Biotinylated ST2485 and BC-2 biodistribution in human tenascin-expressing tumor-transplanted nude mice. The antibody amount is expressed as percent of the injected dose per tissue gram (% I.D./gr).



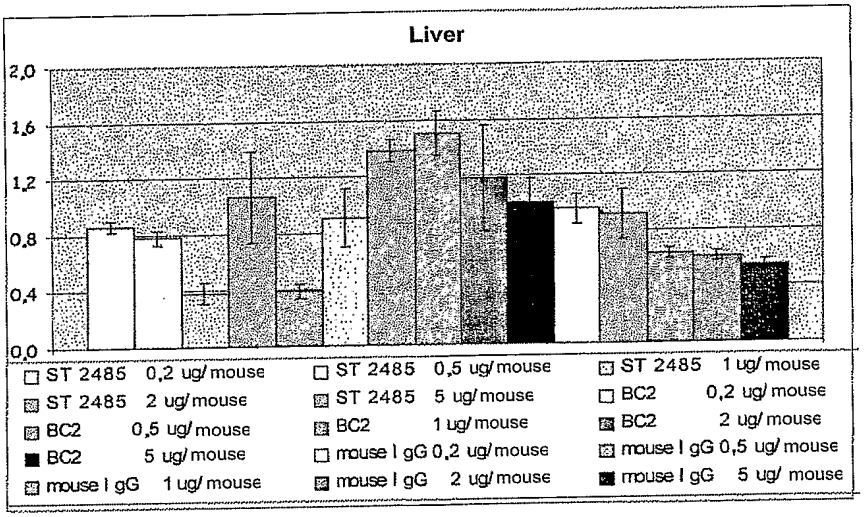
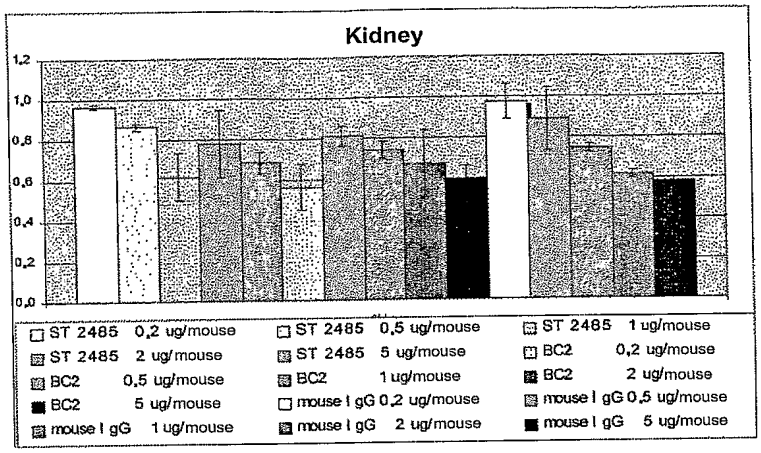
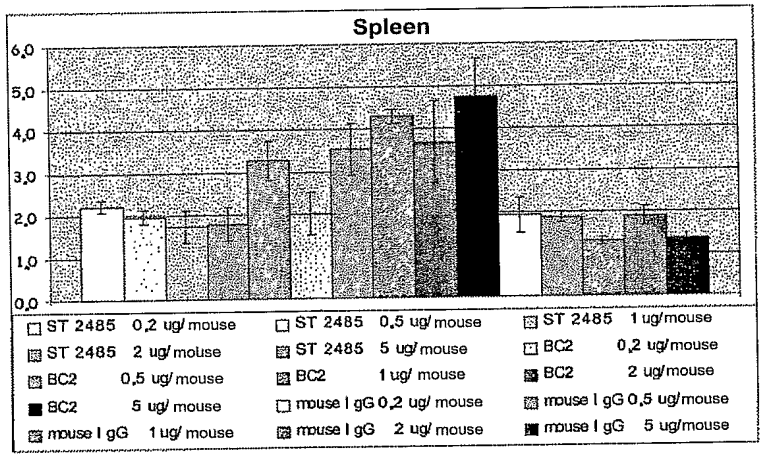


Figure 11a


Kidney
1,2
1,0
0,8
0,6
0,4
0,2
0,0
ST 2485 0,2 ug/mouse
ST 2485 0,5 ug/mouse
ST 2485 1 ug/mouse
ST 2485 2 ug/mouse
ST 2485 5 ug/mouse
BC2 0,2 ug/mouse
BC2 0,5 ug/mouse
BC2 1 ug/mouse
BC2 2 ug/mouse
BC2 5 ug/mouse
mouse I gG 0,2 ug/mouse
mouse I gG 0,5 ug/mouse
mouse I gG 1 ug/mouse
mouse I gG 2 ug/mouse
mouse I gG 5 ug/mouse


Spleen
6,0
5,0
4,0
3,0
2,0
1,0
0,0
ST 2485 0,2 ug/mouse
ST 2485 0,5 ug/mouse
ST 2485 1 ug/mouse
ST 2485 2 ug/mouse
ST 2485 5 ug/mouse
BC2 0,2 ug/mouse
BC2 0,5 ug/mouse
BC2 1 ug/mouse
BC2 2 ug/mouse
BC2 5 ug/mouse
mouse I gG 0,2 ug/mouse
mouse I gG 0,5 ug/mouse
mouse I gG 1 ug/mouse
mouse I gG 2 ug/mouse
mouse I gG 5 ug/mouse

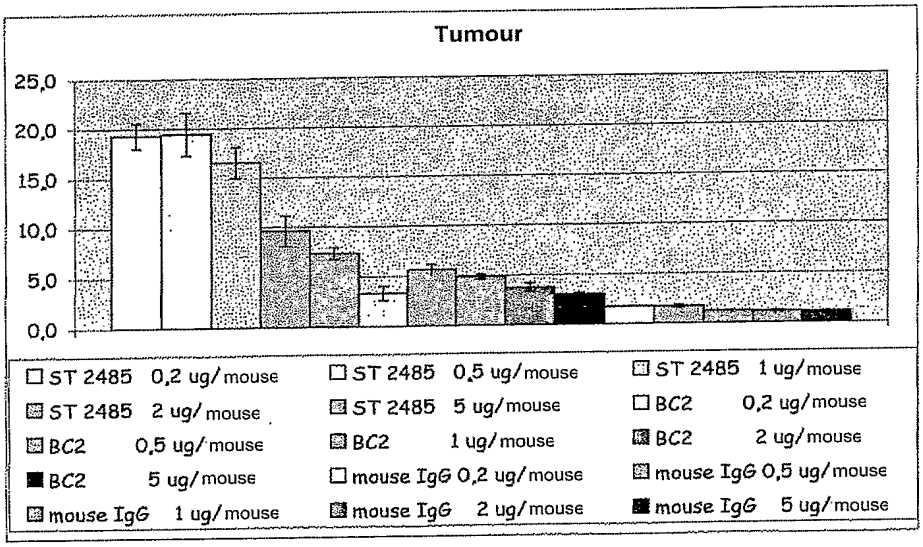
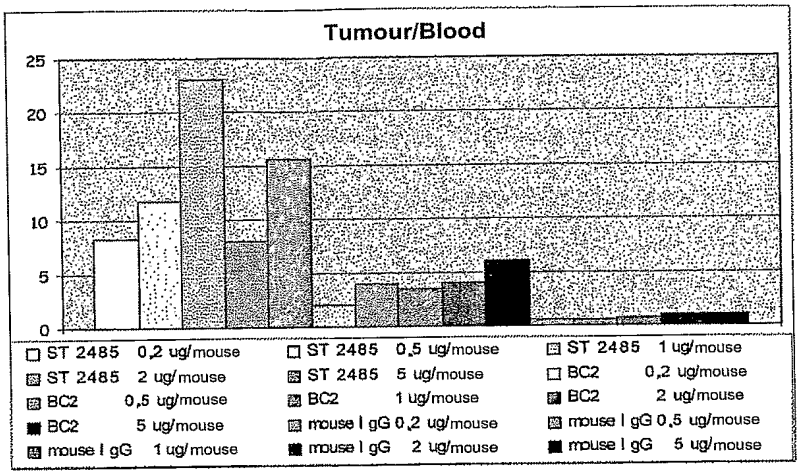

Tumour
25,0
20,0
15,0
10,0
5,0
0,0
ST 2485 0,2 ug/mouse
ST 2485 0,5 ug/mouse
ST 2485 1 ug/mouse
ST 2485 2 ug/mouse
ST 2485 5 ug/mouse
BC2 0,2 ug/mouse
BC2 0,5 ug/mouse
BC2 1 ug/mouse
BC2 2 ug/mouse
BC2 5 ug/mouse
mouse IgG 0,2 ug/mouse
mouse IgG 0,5 ug/mouse
mouse IgG 1 ug/mouse
mouse IgG 2 ug/mouse
mouse IgG 5 ug/mouse

Figure 12

Biotinylated ST2485 and BC-2 biodistribution in nude mice: tumor/non tumor ratio.



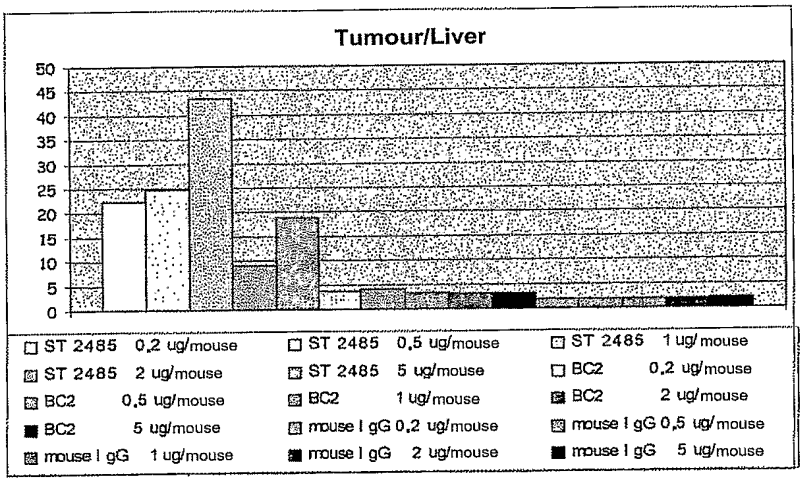
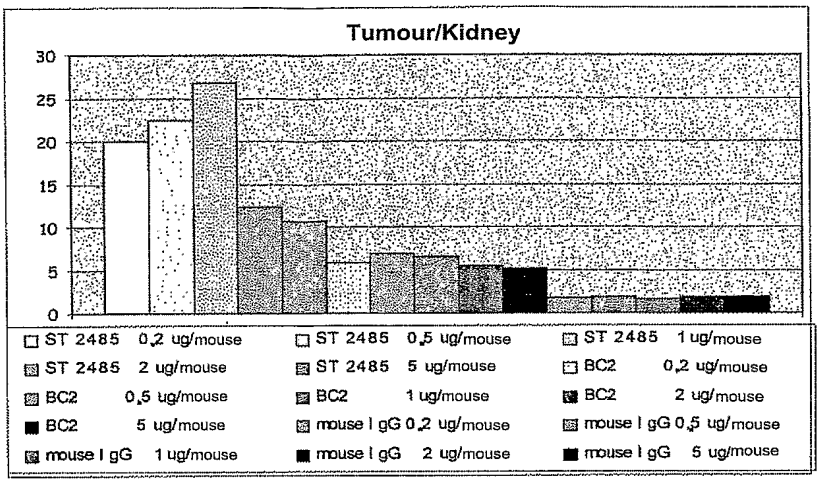


Figure 12a


Tumour/Kidney
30
25
20
15
10
5
0
ST 2485 0,2 ug/mouse
ST 2485 0,5 ug/mouse
ST 2485 1 ug/mouse
ST 2485 2 ug/mouse
ST 2485 5 ug/mouse
BC2 0,2 ug/mouse
BC2 0,5 ug/mouse
BC2 1 ug/mouse
BC2 2 ug/mouse
BC2 5 ug/mouse
mouse I gG 0,2 ug/mouse
mouse I gG 0,5 ug/mouse
mouse I gG 1 ug/mouse
mouse I gG 2 ug/mouse
mouse I gG 5 ug/mouse

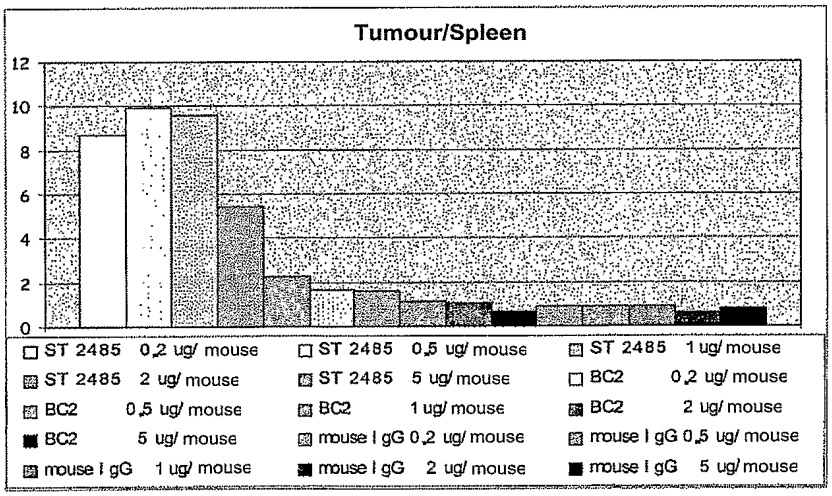
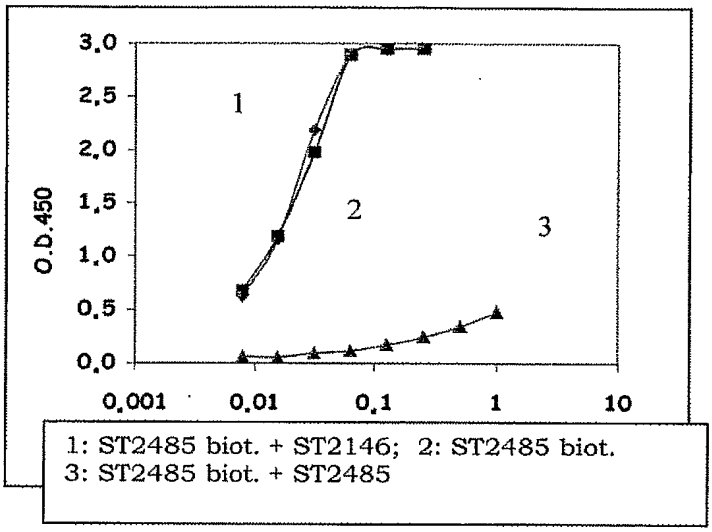

Tumour/Spleen
12
10
8
6
4
2
0
ST 2485 0,2 ug/ mouse
ST 2485 0,5 ug/ mouse
ST 2485 1 ug/ mouse
ST 2485 2 ug/ mouse
ST 2485 5 ug/ mouse
BC2 0,2 ug/ mouse
BC2 0,5 ug/ mouse
BC2 1 ug/ mouse
BC2 2 ug/ mouse
BC2 5 ug/ mouse
mouse I gG 0,2 ug/ mouse
mouse I gG 0,5 ug/ mouse
mouse I gG 1 ug/ mouse
mouse I gG 2 ug/ mouse
mouse I gG 5 ug/ mouse

Figure 13

Interference (a) and additivity (b) ST2485 and ST2146 antitenascin antibodies *in vitro* evaluation by ELISA test.

a) Interference



b) Additivity

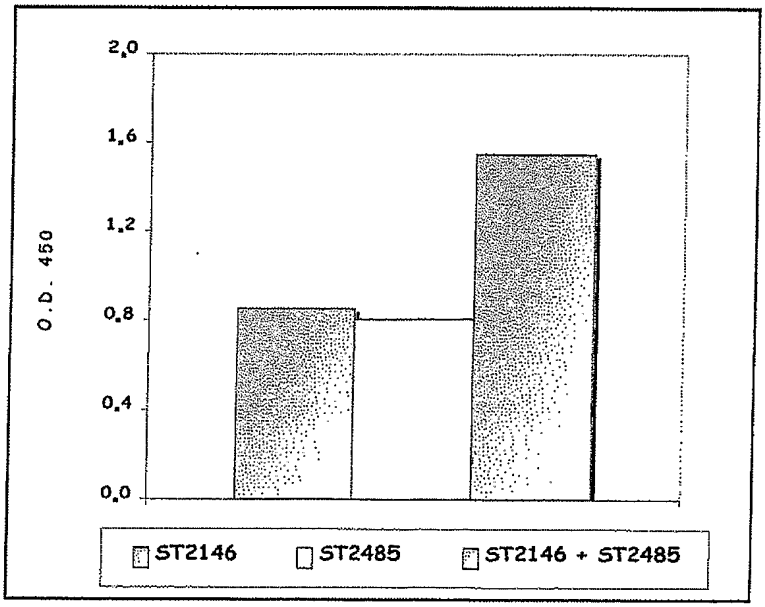
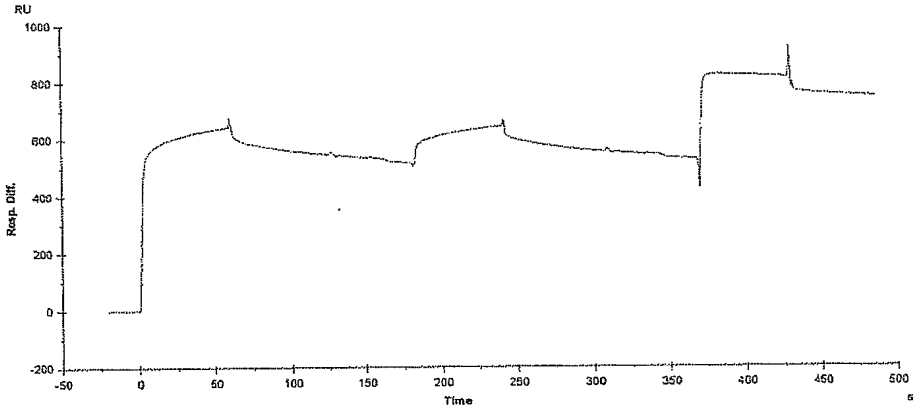


Figure 14

Antibodies ST2485 and ST2146 tenascin binding *in vitro* additivity by BIACore



a)

b)

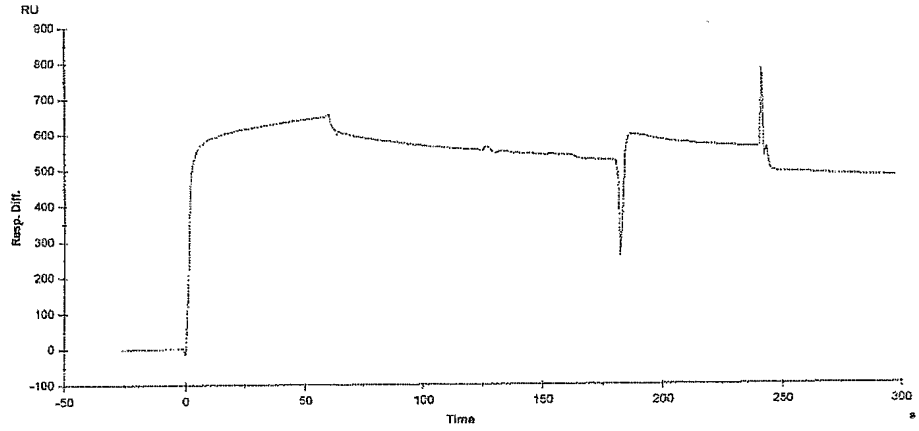
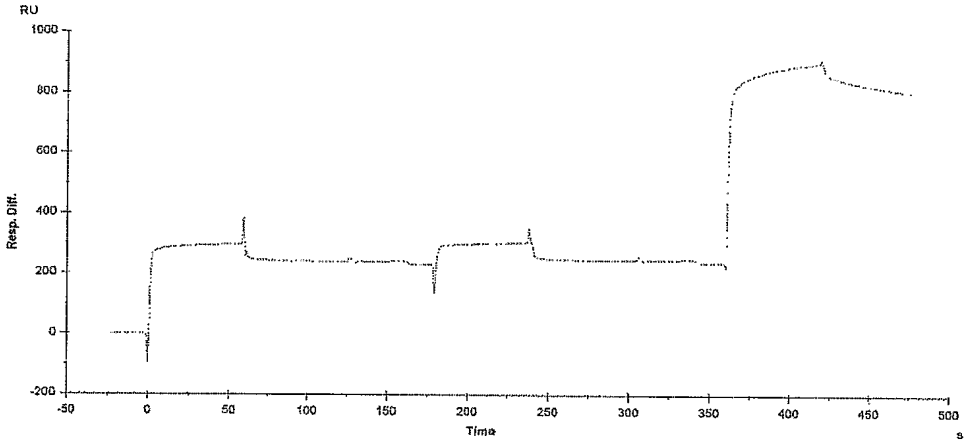
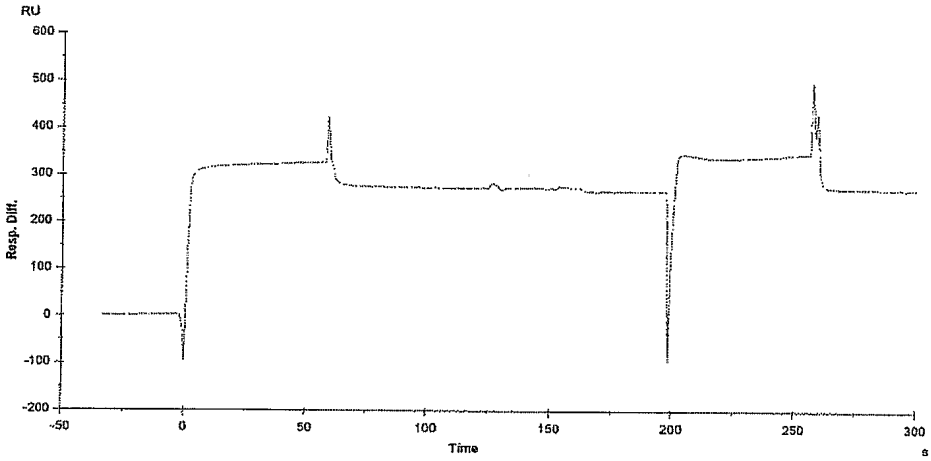


Figure 14a



c)



d)

Figure 15

Schematic representation of *in vivo* additivity study in animal model

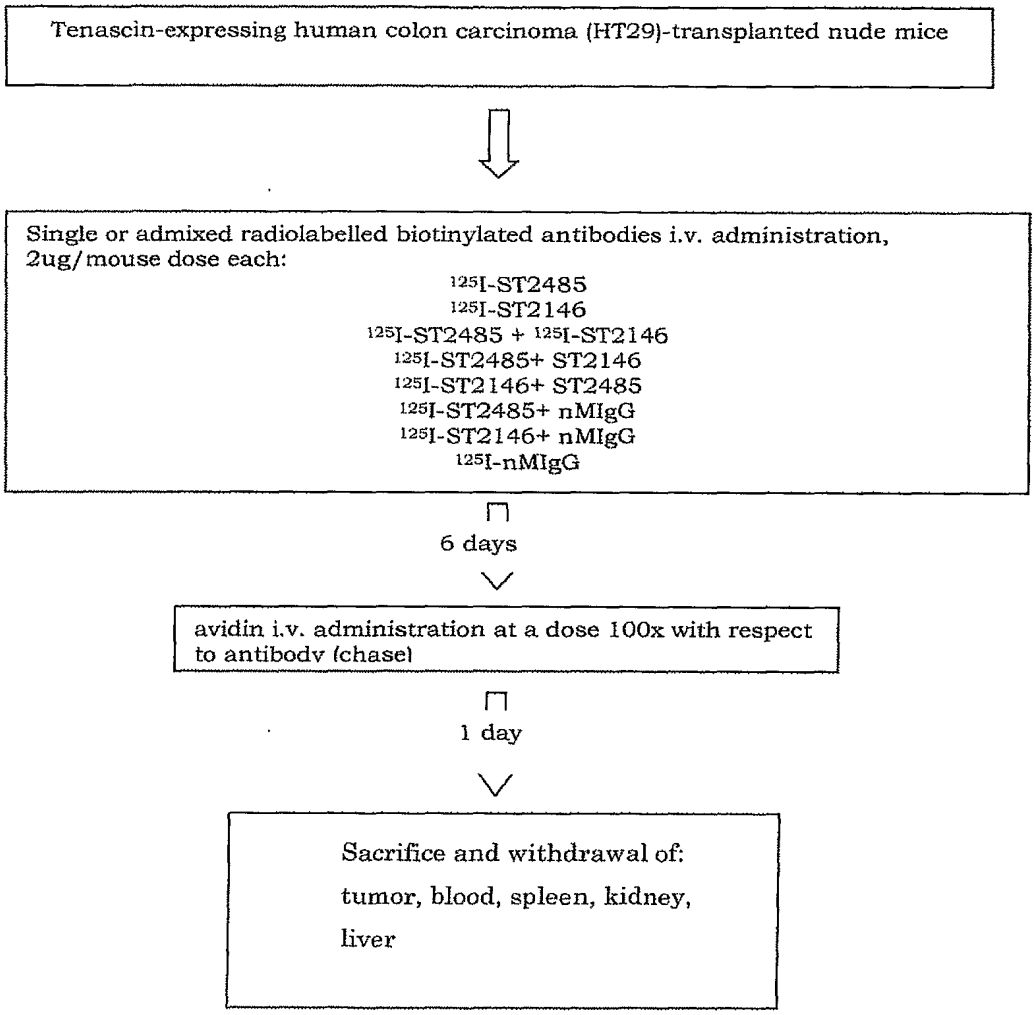


Figure 16

ST2485 e ST2146 antibodies additivity in animal model; tumor seat localization.

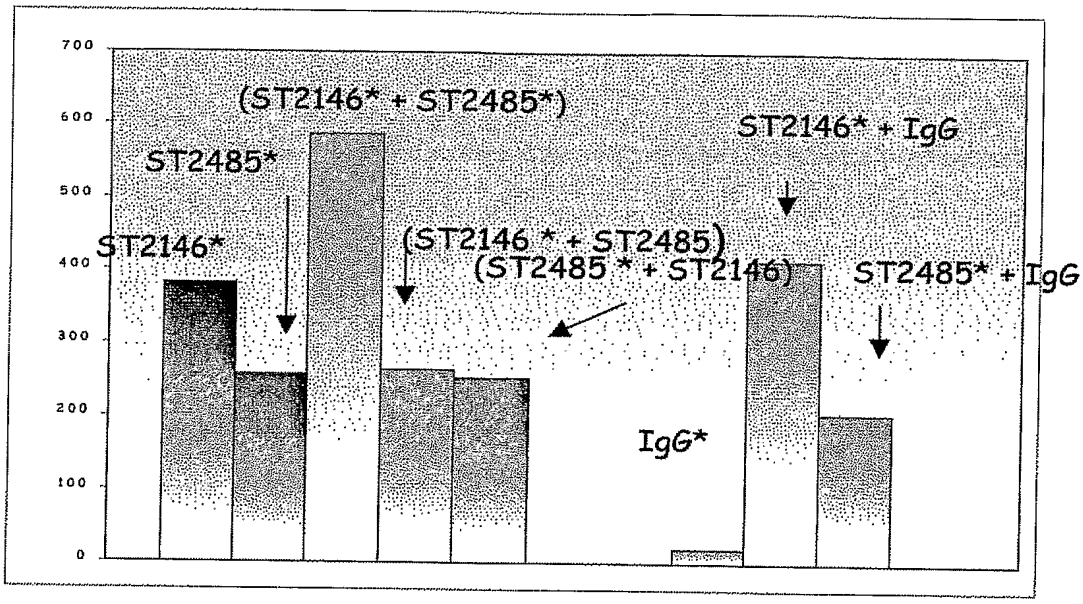


Figure 17

SEQID 1 sequence of ST2485 kappa light chain variable region (VL).

Signal peptide

*ATGGATTTTCAAGTGCAGATTTTCAGCTTCCTGCTAATCAGTGCTTCAGTCATAATGTCCAGAGGA*CAAA
Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser Val Ile Met Ser Arg Gly Gln

TTGTTCTCTCCCAGTCTCCAGCAATCCTGTCTGCATCTCCAGGGGAGAAGGTCACAATGACTTGC
Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys

N-glycosylation

CDR1

AGGGCCAACTCAAGTGTACGTTTCATGCACTGGTACCAGCAGAAGCCAGGATCCTCCCCCAAACC
Arg Ala Asn Ser Ser Val Arg Phe Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys

CDR2

CTGGATTTATGCCACATCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGG
Pro Trp Ile Tyr Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly

CDR3

GACCTCTTATTCTGTCACAATCAGCAGAGTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGC
Ser Gly Thr Ser Tyr Ser Val Thr Ile Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln

AGTGGAGTAGTAATTCACCCAGGACGTTCGGTGGAGGCACCAAGGTGGAAATCAGACGGGCT
Gln Trp Ser Ser Asn Ser Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Arg Arg Ala

Figure 18

SEQID 2 sequence of ST2485 gamma heavy chain variable region (VH)

Signal peptide

*ATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGAACTGCAGGTGTCCACTCT*GAGGTCCAGCTG
Met Gly Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly Val His Ser Glu Val Gln Leu

CAACAGTCTGGACCTGAGCTGGTGAAGCCTGGAGCTTCAATGAAGATTTCCTGCAAGGCTTCTGG
Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Met Lys Ile Ser Cys Lys Ala Ser

CDR1

TTACTCATTCAC[redacted]ACACCATGAACTGGGTGAAGCAGAGCCATGGAAAGAACCTTGAATGGA
Gly Tyr Ser Phe Thr [redacted] Tyr Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu Glu Trp

CDR2

TTGGACTT[redacted]AATCCTCACAATGGTGGTACTACCTACAACCAGAAGTTCAAGGGCAAGGCCACA
Ile Gly Leu [redacted] Asn Pro His Asn Gly Gly Thr Thr Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr

TTAACTGTAGACAAGTCATCCAACACAGCCTACATGGAGCTCCTCAGTCTGACATCTGAGGACTC
Leu Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr Met Glu Leu Leu Ser Leu Thr Ser Glu Asp

CDR3

TGCAGTCTATTACTGTACAAGA[redacted]GGGGGGGTTACTACTGGTTCTTCGATGTCTGGGGCGCAGGGA
Ser Ala Val Tyr Tyr Cys Thr Arg [redacted] Pro Gly Gly Tyr Tyr Trp Phe Phe Asp Val Trp Gly Ala Gly

CCACGGTCACCGTCTCCTCA
Thr Thr Val Thr Val Ser Ser